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FH Valone, PA Kaufman, PM Guyre, LD Lewis, V Memoli, Y Deo, R Graziano, JL Fisher, L Meyer, and M Mrozek-Orlowski

Phase Ia/Ib Trial of Bispecific Antibody MDX-210 in Patients With Advanced Breast or Ovarian Cancer That Overexpresses the Proto-Oncogene HER-2/*neu*

By Frank H. Valone, Peter A. Kaufman, Paul M. Guyre, Lionel D. Lewis, Vincent Memoli, Yashwant Deo, Robert Graziano, Jon L. Fisher, Louise Meyer, Mary Mrozek-Orlowski, Kathleen Wardwell, Veronica Guyre, Theresa L. Morley, Christine Arvizu, and Michael W. Fanger

Purpose: **MDX-210 is a bispecific antibody that binds simultaneously to type I Fc receptors for immunoglobulin G (IgG) (FcγRI) and to the HER-2/*neu* oncogene protein product. MDX-210 effectively directs FcγRI-positive effector cells such as monocytes and macrophages to phagocytose or kill tumor cells that overexpress HER-2/*neu*. The goals of this phase Ia/Ib trial were to determine the maximum-tolerated dose (MTD) and/or the optimal biologic dose (OBD) of MDX-210.**

Patients and Methods: **Patients with advanced breast or ovarian cancer that overexpressed HER-2/*neu* were eligible for treatment. Cohorts of three patients received a single intravenous (IV) infusion of MDX-210 at increasing dose levels from 0.35 to 10.0 mg/m^2.**

Results: **Treatment was well tolerated, with most patients experiencing transient grade 1 to 2 fevers, malaise, and hypotension only. Two patients experienced transient grade 3 hypotension at 10.0 mg/m^2. Transient monocytopenia and lymphopenia developed at 1 to 2 hours, but no other hematologic changes were observed. Doses of MDX-210 ≥ 3.5 mg/m^2 saturated ≥ 80% of monocyte FcγRI and produced peak plasma concentrations ≥ 1 μg/mL, which is greater than the concentration for optimal monocyte/macrophage activation in vitro. Elevated plasma levels of the monocyte products tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and neopterin were observed with maximal levels at doses ≥ 7.0 mg/m^2. Localization of MDX-210 in tumor tissue was demonstrated in two patients. One partial and one mixed tumor response were observed among 10 assessable patients.**

Conclusion: **MDX-210 is immunologically active at well-tolerated doses. The MTD and OBD is 7 to 10 mg/m^2.**

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MANY MONOCLONAL antibodies (mAbs) have been developed to human tumors,[1,2] but despite their specificity for binding to tumors, mAbs have had little direct therapeutic effect. This lack of efficacy results, in part, from the inability of many murine mAbs to activate immune effector pathways such as complement fixation and antibody-dependent cell-mediated cytotoxicity (ADCC) in humans. Many murine mAbs are unable to activate these effector pathways because they are the wrong isotypes. Although humanized mAbs that activate immune effector pathways are being developed, the clinical effectiveness of these mAbs may be impaired by high concentrations of nonspecific immunoglobulins (Igs), which will compete with the mAbs for binding to the high-affinity type I Fc receptors (FcγRI) on immune-effector cells. Moreover, large amounts of immunologically active mAbs may, after complexing with antigen, be directed to Fc receptors on cells (eg, platelets) that are not cytotoxic for tumor cells.

Bispecific mAbs (BsAbs) are one approach to increasing the immunologic effectiveness of therapy with mAbs. BsAbs are hybrid antibodies constructed from two parent mAbs: one specific for the tumor target cell and the other specific for the immune-effector cells.[3,4] BsAb-mediated cross-linking of tumor target cells to immune effector cells via specific cytotoxic trigger molecules on the effector cell, such as FcγRI, results in efficient destruction of the tumor target. BsAbs can direct the cytotoxic activity of monocytes,[3,4] monocyte-derived macrophages, T cells,[5-7] natural-killer (NK) cells,[8,9] and neutrophils[10] to kill and/or ingest tumor target cells in vitro and in vivo.

BsAb MDX-210 is constructed from mAb 22, which recognizes FcγRI,[11,12] and from mAb 520C9, which recognizes the cell-surface protein product of the oncogene HER-2/*neu* (c-*erb*B2).[13] FcγRI is expressed mainly by monocytes and macrophages, but also by neutrophils after activation by interferon gamma (IFNγ) or granulocyte colony-stimulating factor (G-CSF).[10,12,14] mAb 22 is a murine mAb that reacts with FcγRI at a site distinct from its binding site for human IgG.[11,12] Thus, binding of mAb 22 to FcγRI is not inhibited by human IgG. Although

From the Departments of Medicine, Physiology, Microbiology, Pathology, and Nursing, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH; and Medarex, Inc, Annandale, NJ.

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Address reprint requests to Frank H. Valone, MD, Activated Cell Therapy, 219 N Bernardo Ave, Mountain View, CA 94043.

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mAb 22 binds to an epitope distinct from the receptor's ligand-binding site, this mAb still mediates FcγRI-dependent functions, including ADCC, phagocytosis, superoxide generation, and enzyme release.

HER-2/*neu* is a human proto-oncogene homologous to the rat *neu* oncogene and related in sequence to the epidermal growth factor receptor.[15-17] HER-2/*neu* encodes a 185,000-MW protein with tyrosine kinase activity. This proto-oncogene has transforming activity when it is overexpressed or has certain mutations in the transmembrane domain. Gene amplification and protein overexpression have been demonstrated in a variety of adenocarcinomas, including approximately 30% of patients with newly diagnosed breast or ovarian carcinomas. Overexpression may correlate with development of metastases and a poor prognosis.

We completed a phase Ia/Ib trial of a single intravenous infusion of MDX-210 for treatment of patients with advanced breast or ovarian carcinomas that overexpress HER-2/*neu*. The objectives of the study were to define the tolerability and safety of single doses of MDX-210, to find a maximum-tolerated dose (MTD), and to find an optimal, immunologically active dose. We provide evidence in this report that MDX-210 was well tolerated and effective for stimulating immune-effector cells in vivo.

PATIENTS AND METHODS

Patients

Patients with stage IV breast cancer or stage III or IV ovarian cancer were eligible for treatment if their tumors were shown to overexpress HER-2/*neu*. Expression of HER-2/*neu* was determined on paraffin-embedded tissues using standard immunohistochemical methods.[18] Tumors were considered to overexpress HER-2/*neu* if more than 50% of tumor cells stained with moderate to strong intensity. Both 520C9, the anti-HER-2/*neu* mAb component of MDX-210 and a commercially available anti-HER-2/*neu* mAb[19] were used for immunohistochemistry. To be eligible, patients with breast cancer must have failed to respond to at least two standard chemotherapy regimens or one hormonal and one chemotherapy regimen. Patients with ovarian cancer must have failed to respond to at least one platinum-based chemotherapy regimen. Adequate hematologic (WBC count > 3,000/μL, absolute neutrophil count [ANC] > 1,500/μL, platelet count > 150,000/μL), hepatic (bilirubin level < 1.5 mg/dL, ALT and alkaline phosphatase level less than two times normal upper limits), and renal (creatinine concentration < 1.5 mg/dL) function were required. An Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 and anticipated survival time more than 3 months were required. Patients with inflammatory diseases such as lupus, active infections, or addictive or psychiatric disorders that would preclude giving informed consent were excluded. This protocol was approved by the Dartmouth-Hitchcock Medical Center Institutional Review Board. All patients gave written, informed consent before being treated.

Preparation of MDX-210

MDX-210 was prepared using the method reported by Glennie et al.[20] The preparation and characterization of the biologic activities of MDX-210 are described elsewhere (Graziano R, Fisher J, Valone FH, et al, manuscript in preparation). Briefly, the $F(ab')_2$ fragments of murine mAbs 22 (anti-FcγRI) and 520C9 (anti-Her-2/*neu*) were reduced to Fab' by incubation with 10 mmol/L of mercaptoethanolamine. The Fab'-sulfhydryl fragments were conjugated using o-phenylenedimaleimide, acetylated with iodoacetamide, and purified by chromatography on Superdex 200 (Pharmacia, Piscataway, NJ). The binding activity of the individual components of the bispecific antibody was checked by fluorescence-activated cell-sorter (FACS) analysis[21] using the Her-2/*neu*–expressing cell line SKBR3,[22] FcγRI-expressing human monocytes, and U937 cell line[23] as target cells. In addition, the bispecific nature of the molecule was verified by a cell-based bispecific enzyme-linked immunosorbent assay (ELISA). This assay detects intact bispecific antibodies that simultaneously bind to HER-2/*neu*–expressing SKBR3 cells and a soluble FcγRI fused to the heavy chain of human IgM.

Administration of MDX-210

Secure venous access was established in each patient and intravenous (IV) fluids, typically normal saline, were administered throughout the treatment and posttreatment observation time. Patients were pretreated with acetaminophen 650 mg orally, diphenhydramine 25 to 50 mg orally or IV, and lorazepam 0.5 to 1.0 mg orally or IV before administration of MDX-210. A test dose of antibody (0.005 to 0.3 mg) was administered IV. Patients who tolerated the test dose without any allergic reactions were given the remaining dose of antibody 30 minutes later. MDX-210 was administered IV at a rate of 6 mg/h, with a maximum infusion duration of 2 hours. Patients' vital signs were monitored every 15 minutes for 2.5 hours after the test dose, hourly for 6 hours, and then every 4 hours for 16 hours. All patients were hospitalized overnight for observation to identify any delayed side effects. Follow-up evaluation occurred on days 2, 3, 4, 8, 15, 29, 57, and 85.

Monitoring of Clinical and Immunologic Outcomes

Toxicity of treatment was scored according to Cancer and Leukemia Group B (CALGB) modified National Institutes of Health (NIH) common clinical trials criteria. Blood was drawn at frequent intervals for monitoring of toxicity and immunologic efficacy. The times for specific tests differ and are described later.

Dose Escalation of MDX-210

The doses of MDX-210 were 0.35, 1.0, 3.5, 7.0, and 10.0 mg/m^2. The dose was escalated for cohorts of three patients until the MTD of MDX-210 or the optimal biologic dose (OBD) was identified. The MTD was defined as the dose that caused ≥ grade 3 toxicity in less than 50% of patients. The OBD was defined as the dose that yielded maximal saturation of monocyte FcγRI, induced optimal release of cytokines (tumor necrosis factor alpha [TNFα], interleukin-6 [IL-6], G-CSF, IL-1α, IL-1β, and neopterin) into plasma, increased expression of human leukocyte antigen (HLA)-DR, and optimally altered subsets of T cells (CD3, CD4, and CD8), B cells (CD20), and NK cells (CD16).

Cytokine Measurements

The cytokines TNFα, IL-1α, IL-1β, and IL-6, and IFNγ and G-CSF were quantified by ELISA (R & D Systems, Minneapolis, MN). Neopterin was quantified by radioimmunoassay (DRG International Inc, Mountainside, NJ). Plasma HER-2/*neu* was quantified by EIA.

Human Antimouse Antibodies

Human antimouse antibodies (HAMA) response to MDX-210 infusion was determined in the plasma samples of treated patients by a specific ELISA method. Alkaline phosphatase–conjugated goat antihuman antibody was used to detect HAMA that was bound to MDX-210–coated microtiter wells. Plasma samples were collected before infusion of MDX-210 (baseline) and 8 and 15 days and 1, 2, 3, and 4 months postinfusion; samples were stored frozen (−70°C) until used for assays. The HAMA levels were expressed as fold increase over the optical density of the preinfusion (baseline) sample.

Each plasma sample that gave a more than twofold increase in optical density over the baseline was also tested in a neutralization assay to determine antigenic specificity of the HAMA. The plasma samples were separately spiked with 100 μg/mL of either MDX-210, 520C9 $F(ab')_2$, 22 $F(ab')_2$, or a mixture of 520C9 $F(ab')_2$ and 22 $F(ab')_2$, and incubated for 30 minutes at room temperature. The treated samples were then assayed for HAMA as described earlier.

Analysis of Plasma MDX-210 Concentrations

Two-milliliter samples of venous blood were collected before infusion, at the end of the infusion, and at points up to 48 hours after infusion of MDX-210. Plasma was separated and stored at −70°C for determination of plasma MDX-210 concentrations. MDX-210 plasma concentrations were determined by two independent assays. The primary assay used for pharmacokinetic purposes was a specific ELISA for murine Ig with a limit of detection of 0.01 μg/mL. In selected cases, a flow-cytometric assay was used to quantify plasma MDX-210 based on binding to its two target molecules, FcγRI and HER-2/*neu*.[21]

Pharmacokinetic analyses were performed on the data for plasma MDX-210 concentration over time using the PC Nonlin version 4.2 pharmacokinetic program (SCI Software, Lexington, KY). Initial IV bolus test doses of MDX-210 (which represented between 2.1% and 11.5% of the total dose) were given to each subject 30 minutes before the infusion. For pharmacokinetic purposes, these were considered part of the total dose given over the infusion period. The pharmacokinetic analysis performed was that for a constant-rate IV infusion with a noncompartmental model (PC Nonlin model 202). For any individual patient, the elimination constant (β) was estimated by linear regression of a minimum of the terminal four plasma concentration-time data points. Clearance (CL) was calculated in the standard fashion (dose divided by the area under the concentration time curve from time 0 to infinity [$AUC_{(0-\infty)}$]) and the volume of distribution ($Vd_{A\infty}$) was then calculated ($Vd_{A\infty} = CL/\beta$). Maximum concentration (C_{max}) and maximum time (T_{max}) were the observed values.

Flow Cytometry

Ten milliliters of heparinized blood was collected at baseline, completion of the infusion of MDX-210, and at other points up to 48 hours after infusion for analysis of changes in selected leukocyte markers and cell-bound MDX-210. Buffy-coat cells were isolated and flow cytometry was performed as described.[21] FcγRI expression on monocytes and neutrophils was determined at all time points. HLA-DR expression on monocytes and expression of markers on T cells (CD3, CD4, and CD8), B cells (CD20), and NK cells (CD16) were determined at baseline and at 24 hours.

To determine the total number of FcγRI expressed by circulating cells, an aliquot of cells at each time point was incubated with a saturating dose of mAb 22 or MDX-210 (20 μg/mL). Cell-bound murine IgG without or with this preincubation was determined by incubation of isolated cells with labeled goat antimouse IgG.

Statistical Analysis

Pretreatment baseline values and posttreatment values were compared by paired *t* tests without adjustment for multiple sampling. *P* values less than .01 were considered statistically significant.

RESULTS

Patients

Fifteen patients were treated with MDX-210. The median age was 47 years (range, 39 to 69). Nine patients had breast cancer and six had ovarian cancer. They were heavily pretreated, having received a median of four (range, one to seven) different chemotherapy or hormonal therapy regimens before receiving MDX-210. All of the patients' tumors overexpressed HER-2/*neu* as determined by immunohistochemistry, with 60% to 100% of cells staining with moderate intensity.

Toxicity of Treatment With MDX-210

Nonhematologic toxicity. Treatment was generally well tolerated (Table 1). Most patients developed low-grade fevers. The mean ± SE baseline temperature was 36.5 ± .1°C and the mean maximal temperature was 37.7 ± .2°C ($P < .0001$). Five patients had maximal temperatures of 38.0 to 39.0°C. The fevers typically developed 3 to 6 hours after the antibody infusion was started and resolved within 8 hours at doses ≤ 3.5 mg/m^2 and within 12 hours at higher doses. Acetaminophen was effective for fever control. Most patients also developed mild systolic hypotension without reflex tachycardia. However, two patients at the 10-mg/m^2 dose developed grade 3 hypotension, which resolved fully within 1 to 4 hours after institution of fluid supplementation. Hypotension occurred within 2 to 8 hours for patients at the lowest doses of MDX-210, whereas the most severe hypotension occurred at 10 to 18 hours for patients at the 10-mg/m^2 level. During the time of hypotension, patients experienced malaise, which they considered less severe than the malaise caused by chemotherapy. Nine patients experienced nausea, but only one vomited. Five patients had onefold to twofold increases in hepatic transaminase lev-

Table 1. Maximum Nonhematologic Toxicity in Patients Treated With MDX-210

	Dose Level (mg/m²)/Toxicity Grade									
	0.03 (n = 3)		1.0 (n = 3)		3.5 (n = 3)		7.0 (n = 3)		10.0 (n = 3)	
	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4
Nausea/vomiting	2	0	2	0	3	0	3	0	3	0
Nausea	1	0	0	0	3	0	1	0	0	0
Hypotension	1	0	2	0	1	0	3	0	1	2
Skin	0	0	0	0	0	0	0	0	0	0
Hepatic	0	0	1	0	0	0	1	0	3	0
Respiratory	0	0	0	0	0	0	1	0	1	0
Genitourinary	1	0	1	0	0	0	0	0	0	0
Musculoskeletal	0	0	0	0	1	0	0	0	0	0
Other	0	0	0	0	1	0	1	0	1	0

els, which were maximal on day 2 to 3 and resolved spontaneously by day 4 to 7. Two patients who had significant pleural effusions and mild dyspnea before treatment developed increased dyspnea within 8 hours after receiving MDX-210. There was no clinical evidence for bronchospasm and chest x-rays showed increased pleural effusions without increased pulmonary infiltrates. Dyspnea resolved spontaneously by hour 8 in one patient and resolved after thoracentesis in the other patient. A therapeutic thoracentesis had been recommended to the latter patient before antibody therapy.

Hematologic toxicity. The number of peripheral-blood monocytes decreased substantially within 1 to 2 hours after the antibody infusion was started ($P < .0001$) and then gradually returned to baseline by hour 24 (Table 2). However, these changes are considered evidence for immunological efficacy and not a sign of hematologic toxicity. Peripheral-blood monocytes are the principal blood cells targeted by MDX-210. The median number of granulocytes decreased slightly 1 to 2 hours after the infusion was started and then rebounded slightly a few hours later, but the differences were not statistically significant. A slight decrease in lymphocytes occurred ($P < .01$). There were no consistent changes in erythrocytes or platelets.

here were no consistent changes in monocyte expression of HLA-DR or expression of markers on T cells (CD3, CD4, and CD8), B cells (CD20), and NK cells (CD16).

Table 2. Hematologic Changes After Treatment With MDX-210

	Hours After Administration					
Variable	0	1	2	4	8	24
Monocytes ($\times 10^{-3}/\mu L$)	0.4	0.1	0.1	0.2	0.3	0.4
PMNs ($\times 10^{-3}/\mu L$)	3.7	3.2	4.9	6.7	6.5	4.6
Lymphocytes ($\times 10^{-3}/\mu L$)	0.7	0.6	0.4	0.4	0.4	0.6
Platelets ($\times 10^{-3}/\mu L$)	261	269	252	260	278	254
Hgb (g/dL)	11.1	10.8	10.6	11.1	10.2	10.3

NOTE. Data are median values at each time point.

Abbreviations: PMNs, polymorphonuclear leukocytes; Hgb, hemoglobin.

here were no consistent changes in monocyte expression of HLA-DR or expression of markers on T cells (CD3, CD4, and CD8), B cells (CD20), and NK cells (CD16).

Pharmacokinetics of MDX-210

Cellular pharmacokinetics. As MDX-210 binds to FcγRI on monocytes, we analyzed the pharmacokinetics of both monocyte-associated and free plasma antibody. At doses ≥ 3.5 mg/m², all monocytes bound MDX-210 at the end of the infusion (Fig 1A). Greater than 80% of monocytes continued to bear MDX-210 48 hours after infusion of the highest doses. MDX-210 saturated available monocyte FcγRI in a dose-related manner, with maximal saturation of 80% to 90% being achieved at doses ≥ 3.5 mg/m² (Fig 1B). At the highest doses, 20% to 40% of monocyte receptors for FcγRI were occupied by MDX-210 48 hours after antibody infusion.

Plasma pharmacokinetics. Plasma MDX-210 concentration-over-time profiles were generated for all patients, except the first three. In patients no. 1 to 3, the measured plasma MDX-210 concentrations apart from the first data point at the end of the infusion were below the level of detection of the assay. Figure 2 shows the plasma MDX-210 concentration-over-time data for each individual patient (in their respective dosing groups) as semilogarithmic plots. Visual inspection of the data for patients no. 4 to 15 showed a single exponential decay of the plasma MDX-210 concentrations, except possibly in patients no. 5 and 6, in whom the number of data points makes any interpretation difficult.

In patients no. 1 and 2, the mean C_{max} was 0.027 μg/mL (range, 0.025 to 0.029) and the median T_{max} was 0.34

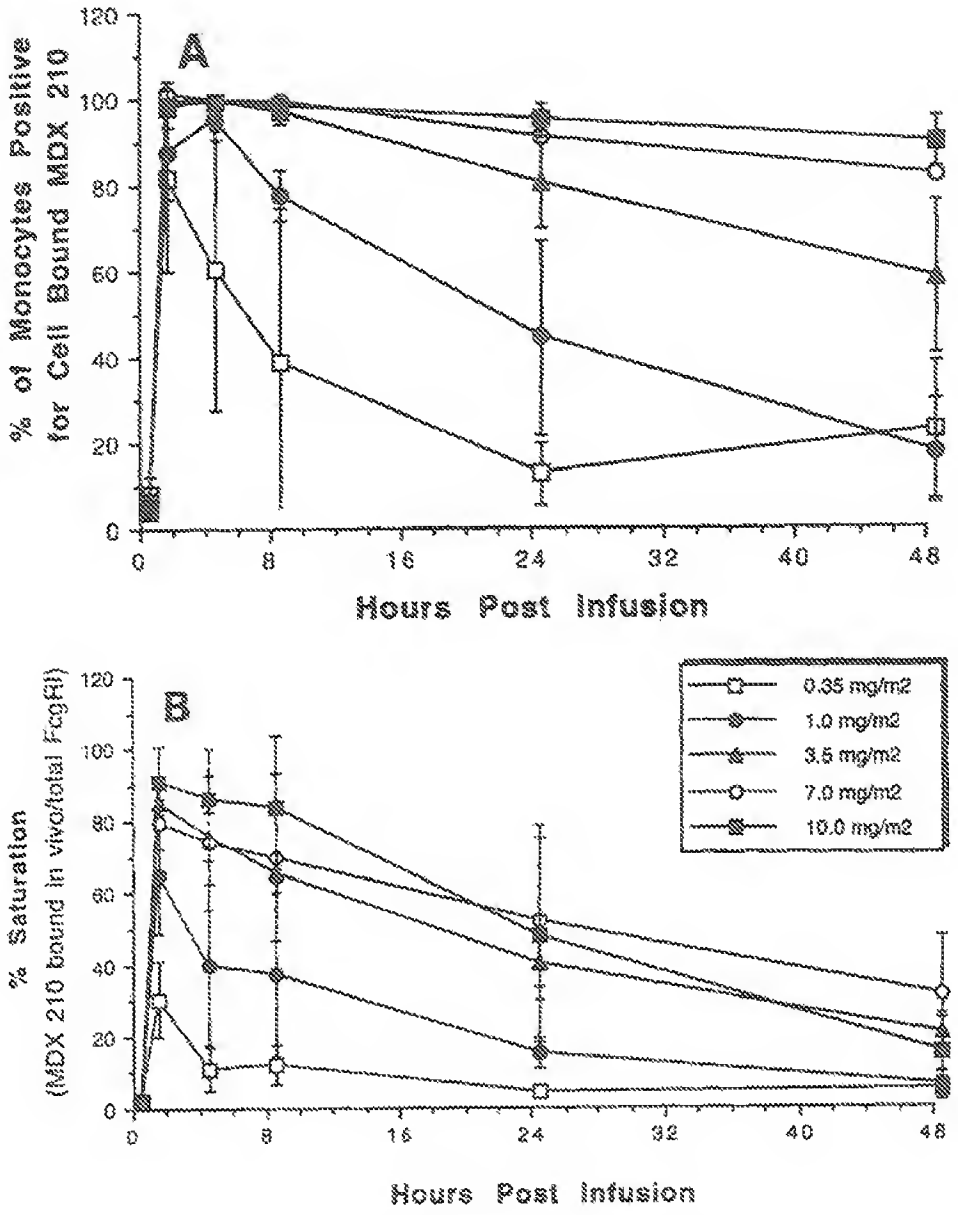

Fig 1. Dose-response and time course of saturation of FcγRI on monocytes by MDX-210 in vivo. Blood was collected at indicated times, and number of molecules of MDX-210 bound to monocytes and total number of FcγRI determined. Three patients were treated at each dose. Data are mean ± SD. (A) Percentage of monocytes bearing MDX-210 at different times postinfusion. (B) Percentage of FcγRI occupied by MDX-210 at different times postinfusion.

hours (range, 0.34 to 0.34). In patient no. 3, the MDX-210 plasma concentrations were below the level of detection of the assay. In the group (patients no. 4 to 6) that received 1.0 mg/m^2 of MDX-210, apart from patient no. 4, there were insufficient measurable MDX-210 concentration-time data points above the level of detection of the assay to define the standard pharmacokinetic parameters accurately. The mean C_{max} for this group was 0.21 μg/mL (range, 0.07 to 0.34), with a median T_{max} of 0.74 hours (range, 0.63 to 0.97). The duration of infusion varied depending on the dose level, but the T_{max} was at the end of or after the end of the infusion in all patients studied. The estimated pharmacokinetic parameters for patients no. 4 and 7 through 15 are listed in Table 3. We found considerable interindividual variation in the estimated pharmacokinetic parameters within any dose group. Because of the small number of patients at each dose level (n = 3), we did not perform statistical analyses for differences in the estimated pharmacokinetic parameters between dose groups.

The mean C_{max} MDX-210 plasma concentration for the three patients treated with 3.5 mg/m^2 (patients no. 7 to

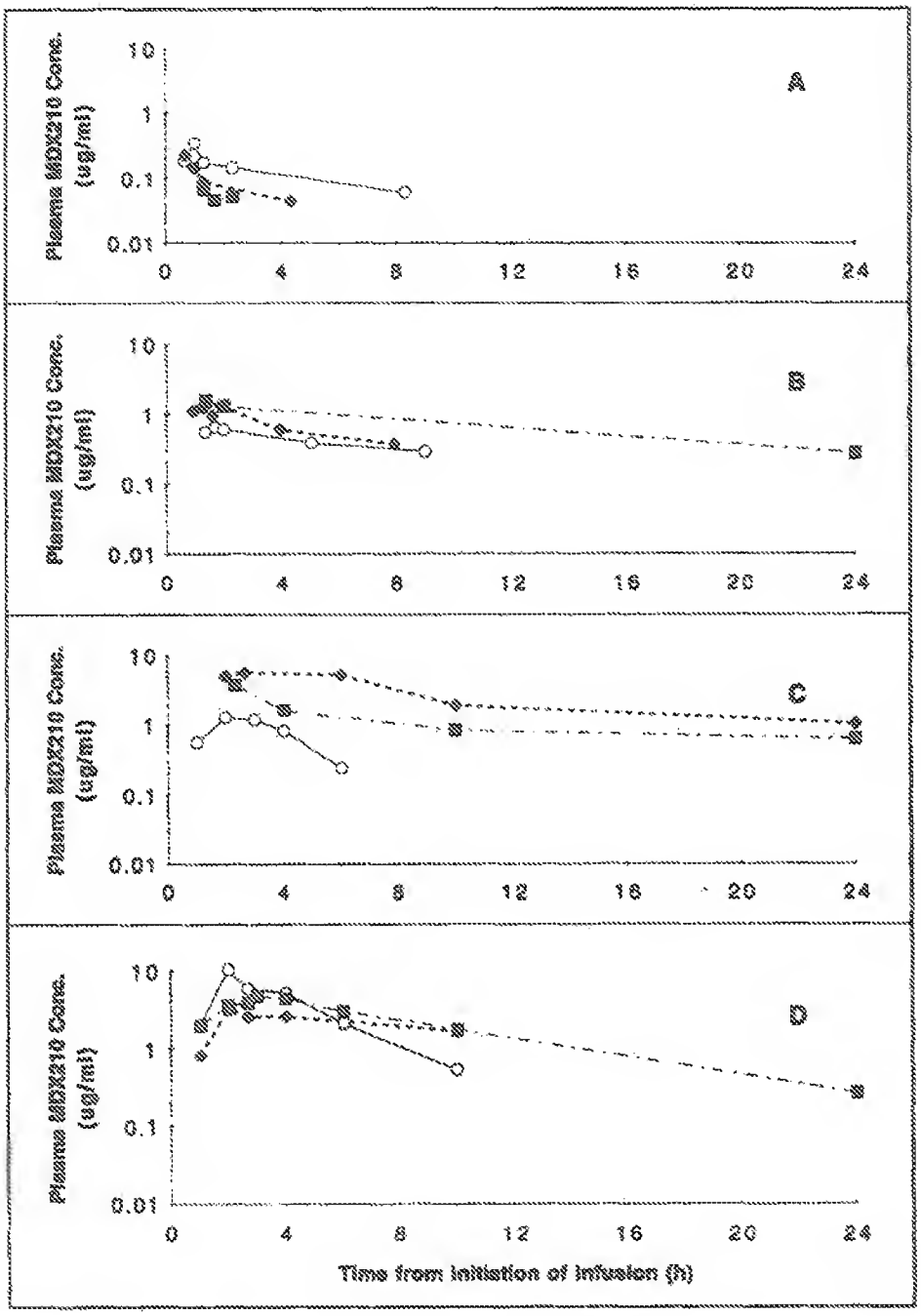

Fig 2. Plasma pharmacokinetics of MDX-210 in patients no. 4 to 15. Plasma concentrations of MDX-210 were too low for patients no. 1 to 3 to permit accurate measurement except at the first time points.

9) was 0.99 μg/mL (range, 0.86 to 1.15) as determined by the ELISA murine IgG assay. The mean C_{max} MDX-210 plasma concentration determined by binding to target antigens was 0.84 μg/mL (range, 0.78 to 0.88), which indicates that in these patients circulating MDX-210 retained its capacity to bind to target antigens.

Immunologic Effects of Treatment With MDX-210

Plasma cytokine concentrations. Plasma concentrations of TNFα, IL-1β, IL-1α, IL-6, neopterin, G-CSF, and IFNγ were determined at baseline and 1, 2, 3, 4, 6, 10, 24, and 48 hours after administration of MDX-210. Treatment with MDX-210 increased plasma concentra-

Table 3. Pharmacokinetic Parameters for MDX-210 After a Single IV Infusion of Between 1 and 10 mg/m²

Patient No.	Dose (mg)	Half-life (hours)	C_{max} (µg/mL)	T_{max} (hours)	$AUC_{0-\infty}$ (µg·h/mL)	Vd_z (L/kg)	Cl (mL/min/kg)
4	1.96	3.95	0.34	0.97	1.35	0.090	0.263
7	5.20	6.08	0.63	1.67	6.43	0.124	0.237
8	6.23	3.74	1.29	1.92	6.76	0.075	0.233
9	5.78	9.42	1.51	1.34	22.93	0.057	0.070
Mean	5.74	6.41	1.15	1.67*	12.04	0.086	0.180
SD	0.52	2.86	0.46		9.44	0.035	0.095
10	10.85	1.61	1.28	2.00	5.12	0.094	0.676
11	10.22	8.79	5.70	2.67	74.16	0.036	0.048
12	13.10	12.58	3.77	2.33	35.47	0.085	0.078
Mean	11.39	7.66	3.59	2.33*	38.25	0.072	0.267
SD	1.51	5.57	2.22		34.60	0.031	0.354
13	17.2	1.92	10.31	2.00	33.83	0.021	0.126
14	18.6	8.84	3.04	2.00	41.51	0.077	0.101
15	16.8	5.41	4.72	3.00	44.71	0.050	0.106
Mean	17.53	5.39	6.02	2.00*	40.02	0.049	0.111
SD	0.95	3.46	3.81		5.59	0.028	0.014

NOTE. Pharmacokinetic results could not be determined accurately for patients no. 1, 2, 3, 5, and 6 because low plasma concentrations of MDX-210 resulted in too few measurements.

*Median T_{max}.

tions of these factors in a complex manner. Increased plasma concentrations of TNFα were observed by hours 1 to 3 in some patients (Table 4). However, only eight patients had increased plasma TNFα levels, and there was no apparent dose response in terms of maximal concentrations of TNFα or the frequency with which elevations were observed. Increased plasma concentrations of IL-6 (Fig 3) and G-CSF (Fig 4) were observed by hours 3 to 6. There appeared to be a threshold effect for stimulation of IL-6 and G-CSF in which low doses of MDX-210 did not stimulate release of these cytokines, but once an active dose was reached, there was no dose-response effect among the active doses. Increased plasma concentrations of neopterin were observed in some patients 24 hours after administration of MDX-210. Plasma neopterin increased in three of three patients at the 3.5-mg/m² dose (mean, 67% increase) and two of three patients at the 7.0-mg/m² dose (mean, 20% increase). In contrast, only one patient at the 10.0-mg/m² level had an increased plasma neopterin level (115% increase). Increased plasma concentrations of IL-1α and IL-1β were not observed for any patient at any time.

Localization of MDX-210 in tumors. Two patients treated at the 10-mg/m² dose level underwent biopsies of skin metastases 24 to 48 hours after treatment. In one patient, a mononuclear-cell infiltrate and associated tumor-cell necrosis were observed (Fig 5A). Immunohistochemical staining showed in vivo binding of MDX-210 to tumor cells in both cases (Fig 5B). Two patients, including one of the patients who underwent a biopsy, de-

Table 4. Plasma TNFα Levels After IV MDX-210

Time (hour)	Patient No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	---	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	115	21	0	0	190	127	0	10	0	213	0	0	0	0	0
2	0	6	0	0	0	0	0	3	0	127	0	12	6	0	0
3	0	0	0	0	0	0	0	0	0	13	0	0	55	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	43	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTE. Data are the means of duplicate determinations and are expressed as pg/mL. Samples with TNFα levels below detection are designated 0.

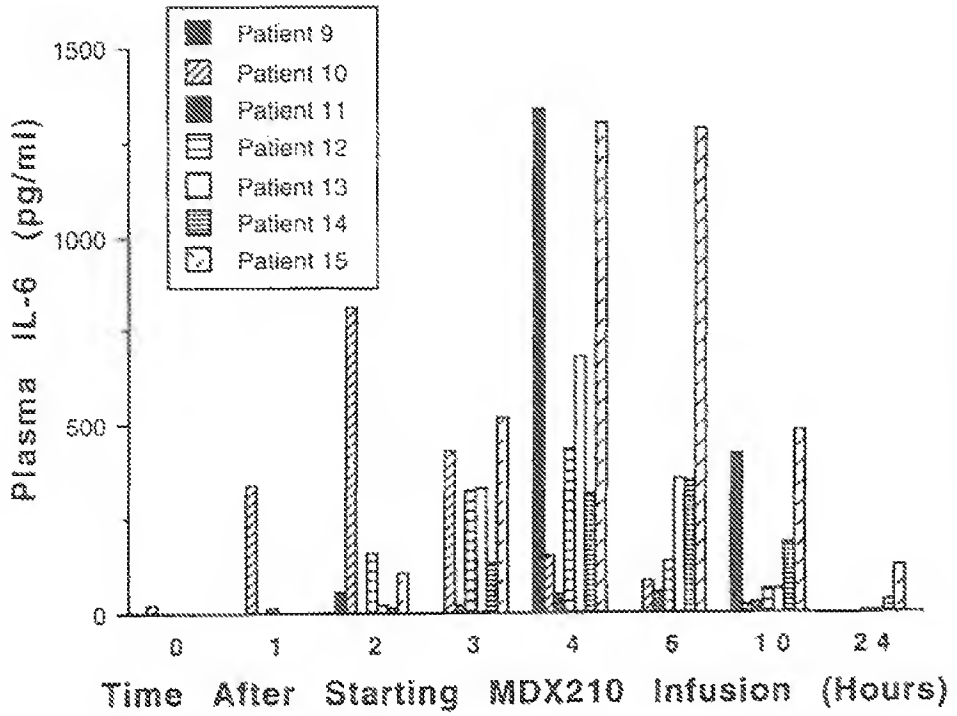

Fig 3. Plasma concentrations of IL-6. Plasma was obtained at the indicated times posttreatment with MDX-210 and the concentration of IL-6 determined by ELISA. Increased plasma levels of IL-6 were found for patients no. 9 to 15. Data are the mean of duplicate determinations for each patient.

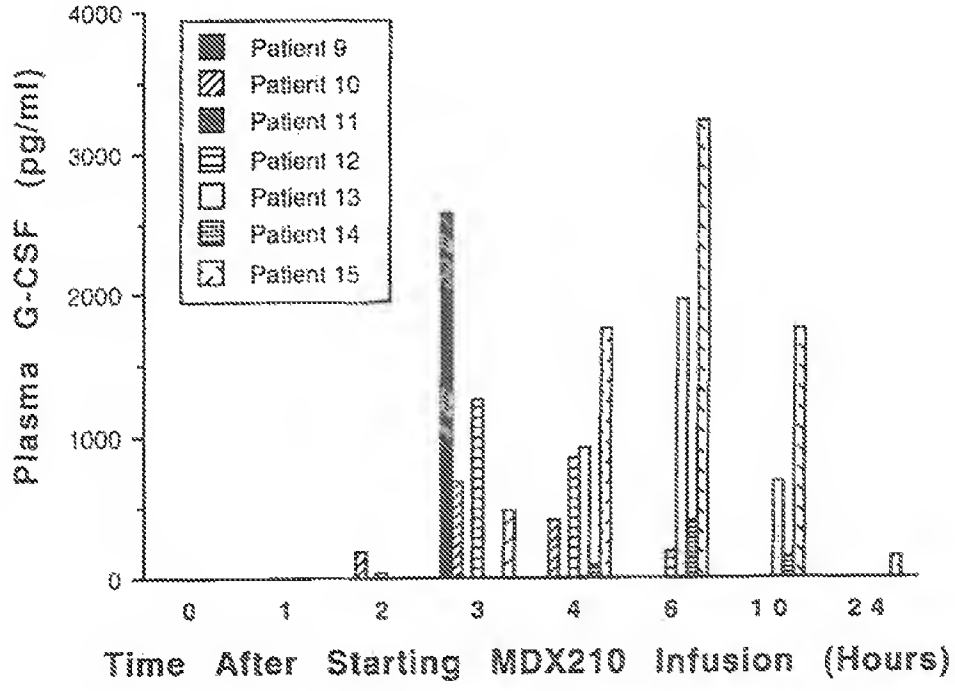

Fig 4. Plasma concentrations of G-CSF. Plasma was obtained at the indicated times and the concentration of G-CSF determined by ELISA. Increased plasma concentrations were observed for patients no. 9 to 15. Data are the mean of duplicate determinations for each patient.

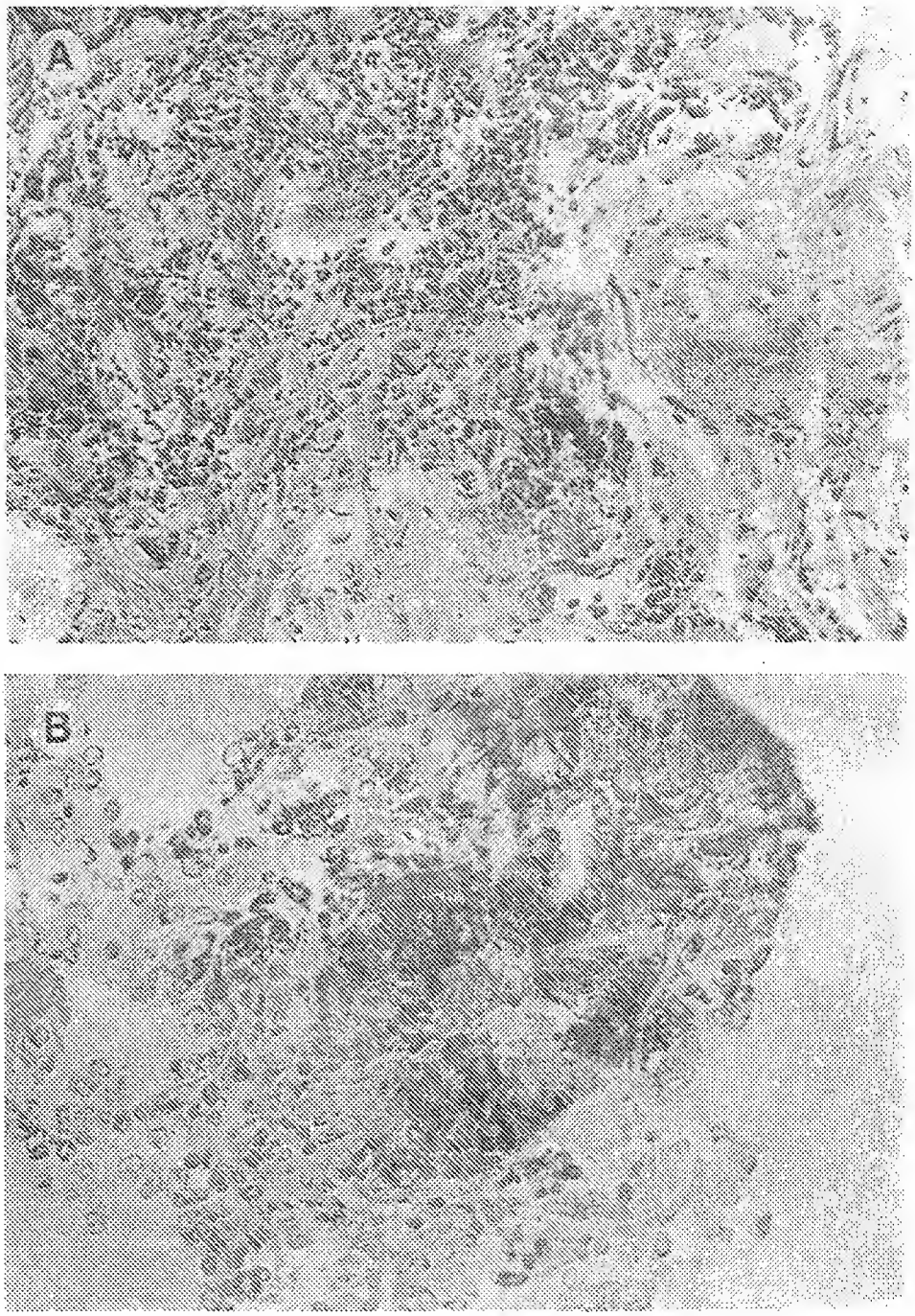

Fig 5. Biopsy of a tumor metastases 48 hours after administration of MDX-210. (A) Hematoxylin and eosin stain demonstrates mononuclear-cell infiltrate and tumor necrosis. (B) Immunohistochemical stain demonstrates localization of MDX-210 in the tumor.

veloped tumor flares with pain at sites of tumor metastases within 4 hours after treatment with MDX-210. The pain resolved by 48 hours after treatment.

Development of Antibodies to MDX-210

HAMA to MDX-210 were measured in plasma samples collected before and after treatment. HAMA were detected in six patients. Development of HAMA appeared to be dose-related, as the number of patients who developed HAMA at the 0.35-, 1.0-, 3.5-, 7.0-, and 10.0-mg/m^2 dose levels were zero, one, one, two, and two, respectively. The plasma concentration of HAMA was generally low. For those patients who developed HAMA, the median

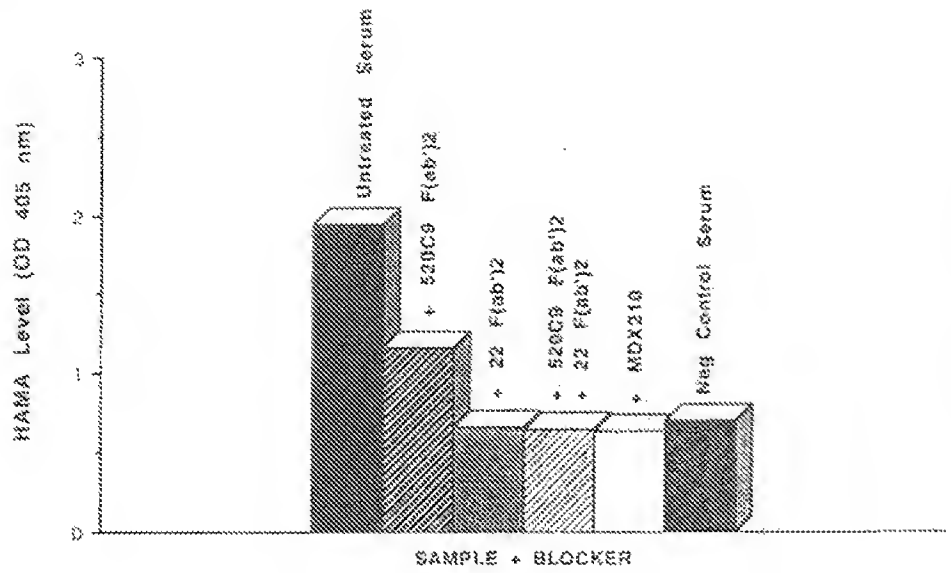

Fig 6. Characterization of HAMA response to MDX-210. Serum was collected from patient no. 15 29 days after treatment with MDX-210. Aliquots were pretreated with the indicated blocker, after which HAMA was quantified. Serum from a subject without HAMA was included as a negative control.

increase in HAMA was 3.5-fold above baseline (range, twofold to fivefold). Data for a representative patient are shown in Fig 6.

To determine what components of MDX-210 (the individual mAbs or the chemical linker) induced HAMA, neutralizing experiments were performed for all patients who had HAMA. $F(ab')_2$ fragments of mAb 22 fully neutralized HAMA, whereas $F(ab')_2$ fragments of mAb 520C9 only partially neutralized HAMA (Fig 6). This suggests that in many patients, HAMA is directed partially to epitopes shared by both mAbs, with the remaining HAMA directed to mAb 22. No specific response to the chemical linker was apparent.

Response to Treatment

Although evaluation of response to treatment was not an objective of this phase I trial, 10 patients were assessable for response. One patient with stage IV breast cancer had a more than 50% decrease in her subcutaneous metastases and axillary adenopathy. She had a tumor flare that began 4 hours after treatment with MDX-210 and lasted for 24 to 48 hours, during which her numerous less than 5-mm subcutaneous nodules and left axillary nodes became erythematous and tender. The adenopathy and all but one of the subcutaneous nodules resolved completely 3 weeks after treatment. However, she had persistent pulmonary infiltrates, consistent with lymphangitic tumor spread. One patient with stage IV ovarian cancer had a more than 50% decrease in cervical adenopathy. However, she developed increased symptoms of bowel obstruction, which suggested that her bulky abdominal masses, which were not measurable, had progressed. The remaining eight patients had no evidence of response to treatment.

DISCUSSION

The goals of this phase Ia/Ib trial were to determine the MTD and/or OBD of MDX-210 when given as a single IV infusion. The main side effects of treatment, fever, hypotension, and malaise are typical of those observed after administration of immunomodulatory cytokines such as IL-2,[24] TNFα,[25] and IL-6.[26] As MDX-210 is designed to stimulate monocyte/macrophage cytotoxicity toward HER-2/*neu*–expressing cells, the development of side effects from recruitment of monocytes and monocyte-derived cytokines may be an unavoidable and perhaps desirable result of treatment. Vascular leakiness induced by cytokines could, for example, improve localization of antibodies and immune-effector cells in tumor tissues. The effects induced by MDX-210 are not typical of those reported for most mAbs, including those that are immunologically active.[27-29] Clinical side effects similar to those induced by MDX-210 have been reported after treatment with bispecific antibodies that target immune-effector cells different from those targeted by MDX-210.[30-32] For example, bispecific antibody 2B1, which targets FcγRIII (CD16) on NK cells, macrophages, and neutrophils, causes fevers and hypotension at doses similar to those used for MDX-210.[30] Dose-limiting toxicity develops at much lower doses of bispecific antibodies that target T cells.[31,32] Although elevated plasma concentrations of TNFα are detected frequently, our experience and that of other groups[33] suggest that increased TNFα concentrations do not necessarily induce toxicity. For MDX-210, the most severe nonhe-

matologic toxicity was related temporally to the maximal plasma concentrations of IL-6, rather than TNFα.

MDX-210 is active immunologically, as evidenced by elevated plasma concentrations of TNFα, IL-6, neopterin, and G-CSF, molecules that are synthesized by monocytes and macrophages.[34-37] Activation of these cells likely accounts for appearance of these cytokines. mAbs that activate monocytes and macrophages in vitro do not usually stimulate cytokine release in vivo.[38,38] This may result from the diminished capacity of mAbs to activate monocytes and macrophages through FcγRI in the presence of excess nonspecific Igs. In contrast, excess nonspecific Ig does not impair immune-effector cell activation by MDX-210.[12]

The MDX-210 plasma concentration-over-time data in the majority of patients suggested a monoexponential plasma decay when MDX-210 was given as a slow (6-mg/h) IV infusion. In contrast, pharmacokinetic analysis of mAbs given as more rapid infusions to humans suggested there was a biexponential decay compatible with a two-compartment model.[39] The longer infusion times in most of our patients (no. 7 to 15) and the sampling times chosen did not enable us to define a two-compartment process. We therefore analyzed our data using a noncompartmental model. In this study, the mean MDX-210 terminal elimination half-life ranged from 3.95 to 7.66 hours, with considerable interindividual variation within each dosing group. The MDX-210 $AUC_{(0-\infty)}$ and total-body clearance also showed considerable interindividual variation within each dosing group. We therefore suggest that these preliminary data are interpreted cautiously. Whole murine mAbs have plasma elimination half-lives of 6 to 48 hours in humans.[2,39] The plasma half-life of MDX-210 is substantially shorter. However, MDX-210 is structurally similar to $F(ab')_2$ fragments of IgG, which have shorter plasma half-lives than that of whole antibodies.[40]

MDX-210 has complex pharmacokinetics and pharmacodynamics because it has two distinct target cells: FcγRI-expressing immune-effector cells and HER-2/*neu*–expressing tumor cells. Doses of MDX-210 ≥ 3.5 mg/m^2 fully saturate FcγRI receptors on circulating monocytes. This observation provides confirmation in vivo that binding of MDX-210 to FcγRI is not blocked by excess nonspecific Ig. A single 3.5-mg/m^2 dose of MDX-210 achieved plasma concentrations more than 0.1 μg/mL for at least 24 hours after treatment. The optimal concentration of MDX-210 for directing monocyte cytotoxicity in vitro is 0.1 μg/mL. Thus, we were able to achieve biologically active plasma concentrations of MDX-210 for sustained periods. We did not examine systematically the localization of MDX-210 in tumors. However, the observations that two patients developed pain at sites of tumor masses and that MDX-210 could be found in both of two tumor specimens obtained 24 to 48 hours after treatment indicate that the antibody localizes in tumor tissue.

The optimal dose of MDX-210 given as a single IV infusion is 7.0 to 10 mg/m^2. As two of three patients had transient grade 3 hypotension at the 10-mg/m^2 dose, the MTD is 7.0 mg/m^2 according to the stringent criteria used in this trial. However, the degree of hypotension experienced at the 10-mg/m^2 dose was tolerable and modest compared with that observed after treatment with cytokines.[24-26] Although cytokine release was comparable at the 7- and 10-mg/m^2 dose levels, it is possible that localization of MDX-210 in tumors is greater at the higher dose. Thus, 10 mg/m^2 may be the OBD.

In conclusion, BsAb MDX-210 is a novel antibody construct designed to direct the cytotoxic effects of monocytes and macrophages via FcγRI to destroy tumor cells that overexpress the HER-2/*neu* protein. This phase Ia/Ib trial of MDX-210 suggests that this agent is well tolerated at doses that appear to be immunologically and clinically active.

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